DPP® SYPHILIS SCREEN and CONFIRM ASSAY

A Unique Point of Care, Single Use Rapid Diagnostic

Rapid Tests for Earlier Treatment
The cause of Syphilis is Treponema pallidum, a spirochete. The Syphilis infection is limited to the human host and is generally transmitted by sexual contact. The disease is spread widely throughout the world. Low concentrations of penicillin can kill T. pallidum, and resistance to penicillin has not been demonstrated.

Approximately 70,000 new Syphilis cases are reported in the U.S. annually; however, the actual number of infections is estimated at 500,000.

The disease manifests itself in infectious lesions on the skin or mucous membranes of the genitalia. In almost 20% of the cases, the primary lesion is intrarectal, perianal or oral, suggesting that T. pallidum can penetrate the intact mucous membranes and skin. Later in the disease, the bacteria multiply at the site of entry, spread to nearby lymph nodes, and reach the bloodstream. In 2 to 10 weeks, a papule develops at the site of infection and breaks down to form an ulcer with a clean, hard base (“hard chancre”). This primary lesion always heals spontaneously, but 2 to 10 weeks later, the secondary lesions appear. Syphilitic meningitis, chorioretinitis, hepatitis, nephritis (immune complex type) or periostitis frequently occur in Syphilis. The Syphilitic infection may remain subclinical and the patient may pass through primary and secondary stages without symptoms and yet develop tertiary lesions. About 30% of cases are cured, 30% remain latent, and 40% progress to the tertiary stage, characterized by granulomatous lesions in skin, bones and liver, degenerative changes in the central nervous system or cardiovascular lesions. However, tertiary Syphilis is not usually infectious.

Syphilis can be transmitted to the fetus through the placenta during the first and second trimesters of gestation. As a result, some infected fetuses can die and miscarry, but some are stillborn at birth, while others are born live, but develop congenital Syphilis or other anomalies.

Public health officials in many countries are attempting to modify current control methods to better identify and treat Syphilis infected prostitutes, drug users, and their sexual contacts based on a dramatic increase of Syphilis rates in many areas.

**SYPHILIS SEROLOGICAL TESTING**

The recommended serological screening algorithm for syphilis involves two types of serologic tests (nontreponemal and treponemal).

**NON-TREPONEMAL TESTS**

Among the non-treponemal tests commonly used are the Venereal Disease Research Lab (VDRL) and rapid plasma reagin (RPR) tests. These tests are based on the fact that particles of the lipid antigen (beef heart cardiolipins) remain dispensed with normal serum, but form visible clumps when combined with reagin. The
results are available in a few minutes and the tests are inexpensive, yet very labor intensive and technique dependent.

Positive VDRL and RPR tests revert to negative in 6 to 18 months after effective treatment. The VDRL can be performed on Cerebral Spinal Fluid (CSF).

Non-treponemal tests are subject to false positives (due to malaria, leprosy, measles, infectious mononucleosis, vaccinations, SLE, rheumatoid arthritis and numerous other conditions), and false negative in tertiary Syphilis. The use of a nontreponemal test alone, such as the rapid plasma reagin (RPR) test, may result in some overtreatment (treatment of uninfected persons) owing to biological false-positive reactions.

TREPOEMAL TESTS

Among the most commonly performed treponemal tests are Fluorescent Treponema Antibody Test (FTA-ABS) and Treponema Pallidum Particle Agglutination Test (TPPA). Newer enzyme immune assays (EIA) and chemiluminescence immunoassay (CLIA) treponemal antibody tests have been introduced in both microplate format, for large volume lab testing (blood bank) as well as a rapid point of care test (POCT).

The treponemal tests show excellent specificity and sensitivity for Syphilis antibodies. In the European Union (EU) the treponemal test is used as the first screen for syphilis. This test, however, cannot be used to judge the efficacy of Syphilis treatment. In addition, the use of treponemal tests alone may also result in overtreatment because they do not differentiate between adequately treated and previous infections.

Treponemal tests have long been considered a confirmatory test that have followed a positive non-treponemal screening test. Positive screening tests are then typically referred for a non-treponemal titer for treatment surveillance and epidemiology purposes.

ALGORITHMS

We will consider three algorithms and their pluses and minuses: the Traditional Algorithm, The Alternate or Reverse Sequence Algorithm and the Rapid Point of Care Algorithm. As mentioned earlier, traditional algorithm, in the USA, has been a specimen received

is screened with a non-treponemal assay, most commonly the RPR, with negatives reported out as non-reactive. Reactive results reflex to a treponemal confirmation, generally the TPPA or EIA.

The advantages of the traditional algorithm is the
Rapid Tests for Earlier Treatment

The majority of tests are normally non-reactive and are culled out with a relatively low cost assay in RPR. The disadvantages to the traditional algorithm are in the labor issues and the methodology issues including specificity challenges. The RPR is a manual method that requires serum separation and heat inactivation, a technician to manually pipette and disburse sample and add reagent, and after a mixing period, read a very subjective result. The RPR procedure should be run under very controlled environmental and lighting conditions that can result in reproducibility issues, technician performance variability, and reading errors.

An additional issue, most important to high volume testing labs, is the labor issue. The RPR being so labor intensive, requires a significant amount of trained technician time as compared to a fully automated assay procedure. There is a large problem with few qualified technicians choosing the laboratory field today as well as many current technicians retiring or moving on to other fields. The result is finding technicians for the lab (at higher labor cost) and the actual tech time involved in RPR testing, has become more expensive and in many cases outweighs the advantage of the less expensive, lower cost RPR assay.

Recently in the USA an Alternate or Reverse Sequence Screening algorithm is being reviewed by several federal agencies and has been instituted in some labs. As mentioned earlier, this is the preferred algorithm in the EU. This alternate algorithm involves performing the Treponemal assay first then reflexing reactive specimens to a non-treponemal assay for confirmation, thus sometimes referred to as the “reverse algorithm”.

The clear advantage of the reverse algorithm is the labor savings. The screening of the samples is done with automated equipment eliminating the bulk of the labor cost.

The drawback on the reverse assay is it determines treponemal immunity status which can appear for the life of a one time infected individual. Thus a positive treponemal test does not indicate an active infection, which can result in misdiagnosis if results are released prior to, or in the absence of, a non-treponemal follow up. This can result in over treatment of antibiotic and patient angst.

The newest arrival is a Rapid Point of Care (POC) Algorithm. With the approval of rapid point of care single use tests, a new paradigm must be considered. The current syphilis POC tests are simple lateral flow devices that utilize a treponemal component. The test upside is that a sample can be run with the patient present, in 15 to 30 minutes, allowing a physician to determine if the patient has been infected with syphilis. The downside of the test is that it does not indicate that a positive test is an active infection and must be reflexed to a traditional nontreponemal assay such as RPR. This would allow a screening of negatives and involves the traditional blood draw, lab submission and extended time for any positive result.

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**FIGURE. CDC recommended algorithm for reverse sequence syphilis screening (treponemal sets screening followed by nontreponemal test confirmation*)**

- **EIA OR CIA**
  - EIA / CIA
    - EIA / CIA
    - Quantitative RPR or other nontreponemal test
      - RPR
        - RPR
          - TP-PA
            - TP-PA
              - TP-PA
                - TP-PA
      - TP-PA
        - TP-PA
          - TP-PA

*) Treponemal sets screening followed by nontreponemal test confirmation.
A new point of care, rapid, single use syphilis test has been developed by Chembio Diagnostic Systems which employs both a Treponemal and Nontreponemal component on their proprietary Dual Path Platform (DPP®).

The Chembio DPP® Syphilis Screen & Confirm Assay IS NOT CURRENTLY CLEARED OR AVAILABLE FOR CLINICAL USE IN THE US.

The Chembio DPP® Syphilis Screen & Confirm Assay IS CE MARKED AND AVAILABLE FOR SALE AND CLINICAL USE OUTSIDE THE US WHERE INDICATED.

The DPP® Syphilis Screen and Confirm Assay (DPPSC) provides a rapid (20 minutes), accurate visual reading at room temperature allowing for the utilization of either the traditional algorithm or the reverse algorithm. The treponemal marker (line) indicates a history of syphilis infection detecting IgG to T. palladium. The nontreponemal marker was developed at the US Center for Disease Control and has been licensed for use in the DPP® format by Chembio Diagnostic Systems.

Considering the presence of both the treponemal and non-treponemal markers on the dual syphilis POC assay will permit the clinician to initiate treatment and partner notification activities at the consultation site. Statistics have shown that in outreach and clinical settings as many as 40 to 50 percent of those testing for STDs never return for test results that traditionally have taken from 24 to 48 hours to turn around. A rapid dual syphilis test could greatly improve efforts to control and eradicate syphilis.

In addition to clinical and outreach use the DPP® Syphilis Screen and Confirm will be of utility in the lab setting as a follow up to a syphilis positive screening. In either the traditional or alternate algorithm the suggested next step is to run the complementary assay to the Treponemal or non treponemal screen. The DPPSC will allow for that follow-up step and a repeat of the screening result effectively and efficiently. This will be a welcome addition to most small, medium and large laboratories that currently only offer non treponemal screens, or routinely send out specimens for syphilis serology. In addition, the rapid and simple performance characteristics of DPPSC outweigh the cost and labor of running current or traditional methods for Syphilis confirmation.

The DPP® dual POC test exhibits remarkable...
Rapid Tests for Earlier Treatment performance characteristics compared to the standard laboratory-based testing algorithm. When sera have RPR titers of 1:8, the dual POC test has, in preclinical studies, a sensitivity of 99.7%.

**Table 1.** Performance characteristics of the dual POC syphilis test (nontreponemal line) compared to the rapid plasma reagin (RPR) test.

<table>
<thead>
<tr>
<th>Dual test Non-treponemal Line</th>
<th>Number of samples tested with RPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>739</td>
</tr>
<tr>
<td>Negative</td>
<td>95*</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>834</td>
</tr>
</tbody>
</table>

Sensitivity 88.6%, Specificity 98.6%

*Of the 95 samples missed with the dual POC test had a RPR titer of 1:1 with RPR titer ≥ 1:2 the sensitivity was 98.7%

**Table 2.** Performance characteristics of the dual POC syphilis test (treponemal line) compared to the comparator *Treponema pallidum* passive particle agglutination (TP-PA) test.

<table>
<thead>
<tr>
<th>Dual test Treponemal Line</th>
<th>Number of samples tested with TP-PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>972</td>
</tr>
<tr>
<td>Negative</td>
<td>35</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1007</td>
</tr>
</tbody>
</table>

Sensitivity 96.5%, Specificity 95.5%

**Table 3.** Documented cases of syphilis tested with the dual DPP-POC test and the comparator rapid plasma reagin (RPR) and the *Treponema pallidum* passive particle agglutination (TP-PA) tests.

<table>
<thead>
<tr>
<th>Syphilis category</th>
<th>Number of serum samples reactive (Sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dual rapid test</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Primary untreated</td>
<td>7</td>
</tr>
<tr>
<td>Primary treated</td>
<td>14</td>
</tr>
<tr>
<td>Secondary untreated</td>
<td>6</td>
</tr>
<tr>
<td>Secondary treated</td>
<td>28</td>
</tr>
<tr>
<td>Latent untreated</td>
<td>5</td>
</tr>
<tr>
<td>Latent treated</td>
<td>45</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>105</td>
</tr>
</tbody>
</table>

*aNon-Trepa, Nontreponemal Line  bTreponemal Line*
World Health Organization 2011 New Syphilis Cases Yearly Estimates

Access to syphilis diagnostics is limited in regions of high disease burden

REFERENCES


